

# Multiple factor analysis of the action of local anesthetics

(anesthesia/synergism/summation/antagonism/rates of change)

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**ABSTRACT** The pH jump data of Bianchi and Strobel [(1968) *Trans. N.Y. Acad. Sci. Ser. II*, 30, 1082-1092] on desheathed frog sciatic nerve are fitted to rate equations. A general quantitation of synergism, summation, and antagonism of anesthetics and of excitation is given.

Clinically used local anesthetics possess hydrophobic benzene at one end and tertiary amine at the other. The tertiary amine end is protonated to form strongly hydrophilic quaternary amine, depending upon the pH of the medium and  $pK_a$  of the compound. The values of  $pK_a$  of clinical local anesthetics lie between 7.5 and 9. At physiological pH they exist as both positively charged and uncharged molecules. It has been argued as to which is the biologically active form.

Skou (1) demonstrated, with frog sciatic nerve, that the nerve blocking activity of dissociable local anesthetics is increased in alkaline medium and concluded that uncharged molecules are the active species. This idea was challenged by Ritchie and Greengard (2), who demonstrated with desheathed rat vagus nerve that the change of pH of the bathing medium from 9.6 to 7.2 increased the blocking activity and the change from 7.2 to 9.6 decreased it. From this result they postulated that the uncharged form is required for penetration of the drug through the membrane and that the drug binds to the membrane in the charged form from inside of the membrane. Narahashi and coworkers (3), using internally perfused squid giant axon, found that when procaine is administered from the axoplasmic side of the cell, the blocking potency was larger at the lower pH range. They maintain that local anesthetics bind to the nerve cell membrane from the axoplasmic side in charged form.

However, the fact that uncharged molecules, like alcohol and general anesthetics, also act as local anesthetics is difficult to reconcile with the idea that only charged molecules are the active species. A clinically used local anesthetic, benzocaine, is also devoid of electrical charge and shows nerve blocking activity.

Nishimura *et al.* (4) demonstrated, with isotope-labeled local anesthetics and crystalline bovine serum albumin, that the binding of these agents is higher in the high pH range than in the low pH range. A sharp change in binding was observed with each anesthetic at a pH value close to the  $pK_a$  of the compound. The isoelectric point of bovine serum albumin is pH 4.9, and this protein is negatively charged throughout the pH range for which the binding study was performed. In spite of the surface negative charge, uncharged local anesthetics are preferred for binding over the positively charged species. Their study indicates that local anesthetics appear to interact with nerve cell membranes hydrophobically.

Bianchi and Strobel (5) demonstrated, with desheathed frog sciatic nerve, that the blocking action of local anesthetics increased after a change of pH from 9.2 to 7.2, but this increased

blocking activity was transitory and returned to the level of pH 9.2 and decreased further when enough time was allowed to elapse. The model was similar to the one used by Ritchie and Greengard (2). They proposed that uncharged species of local anesthetics binds to the cell membrane, and protonation of the compound in the membrane by the pH jump increases the blocking activity. The increased blocking action is reversed during the time course because the protonated molecules are not favored to adhere to the membrane.

Our study with a firefly luminescent system (6) showed that the inhibitory action of dissociable local anesthetics on this bioluminescence is higher in the alkaline range, suggesting that the uncharged species is more active. We also found that the charged molecule has some effect, and speculated that neutralization of the surface negative charge of the light-emitting enzyme by the positive charge of the bound local anesthetic would enhance the inhibitory action.

We propose that there are at least two species of local anesthetics that are involved in the depression of nerve activity. In this communication, a generalized equation for inhibition of nerve activity by multi-factors is presented and is used to fit the pH jump data of Bianchi and Strobel (5).

## THEORY

Local anesthetics (A) work on receptor sites (S) of nerves to inhibit nerve activity (AS).



Let  $\sigma$  represent the fraction of nerve receptor site that is inhibited. The reaction rate of the system can then be expressed by the equation

$$\frac{d\sigma}{dt} = k_1 c(1 - \sigma) - k_2 \sigma \quad [2]$$

where  $c$  denotes the concentration of the local anesthetics causing the effect.

Eq. 2 was solved as follows,

$$\sigma = \frac{1}{(ck_1 + k_2)} \{ck_1 + e^{-(ck_1 + k_2)(t - \tau)}\} \\ \equiv \frac{B}{A} - \frac{1}{A} e^{-A(t - \tau)} \quad [3]$$

where parameters  $A$  and  $B$  are  $(ck_1 + k_2)$  and  $ck_1$ , respectively, and  $\tau$  is the time when  $\sigma$  reaches the value  $(B - 1)/A$ .

Eq. 2 represents each of the mechanisms inhibiting nerve action potential. Eq. 3 can be generalized to take account of more than one effect as follows,

$$\sigma = \sum_i \pm F_i \left\{ \frac{B_i}{A_i} - \frac{1}{A_i} e^{-A_i(t - \tau_i)} \right\} \quad [4]$$

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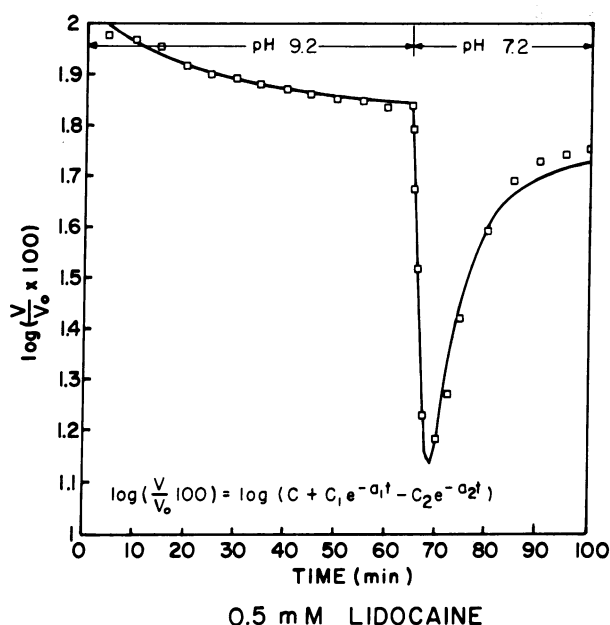


FIG. 1. Curve fitting of the action of lidocaine reported by Bianchi and Strobel (5).

The percentage of the action potential at time  $t$  is

$$V/V_0 \times 100 = (1 - \sigma) \times 100$$

$$\equiv 1 - \sum_i \pm F_i \left\{ \frac{B_i}{A_i} - \frac{1}{A_i} e^{-A_i(t - \tau_i)} \right\} \times 100 \quad [5]$$

where  $V$  is the size of the action potential at time  $t$  and  $V_0$  is its value at  $t = 0$ . In Eq. 5,  $F_i$  is the fractional contribution of each mechanism in the summation.

### APPLICATION

The results of Bianchi and Strobel (5) on the depression of the nerve action potential of the desheathed frog sciatic nerve by 0.5 mM lidocaine and procaine were analyzed using Eq. 5.

Eq. 5 was used in the form

$$\begin{aligned} \frac{V}{V_0} \times 100 &= \left\{ 1 - \left[ F_1 \left( \frac{B_1}{A_1} - \frac{1}{A_1} e^{-A_1(t - \tau_1)} \right) \right. \right. \\ &\quad \left. \left. - F_2 \left( \frac{B_2}{A_2} - \frac{1}{A_2} e^{-A_2(t - \tau_2)} \right) \right] \right\} \times 100 \\ &= G + D e^{-A_1(t - \tau_1)} - E e^{-A_2(t - \tau_2)} \quad [6] \end{aligned}$$

where parameters  $G$ ,  $D$ , and  $E$  are  $(1 - F_1 B_1/A_1 + F_2 B_2/A_2) \times 100$ ,  $F_1 \times (100/A_1)$ , and  $F_2 \times (100/A_2)$ , respectively.

The curves were fitted by Eq. 7 with a Hewlett Packard 9810A using a program for nonlinear least square curve fitting (Figs. 1 and 2).

$$\log(V/V_0 \times 100) = \log \{ G + D e^{-A_1(t - \tau_1)} - E e^{-A_2(t - \tau_2)} \} \quad [7]$$

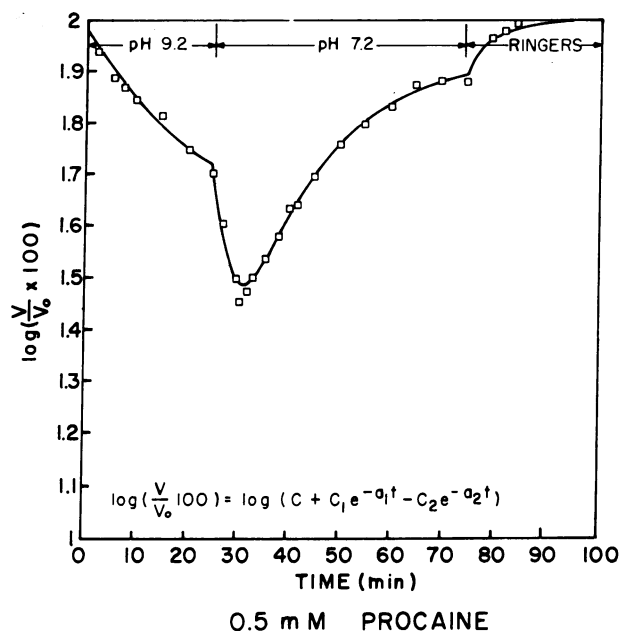


FIG. 2. Curve fitting of the action of procaine (5).

Seven parameters occur naturally in the curve fitting analysis. They are  $A_1$ ,  $A_2$ ,  $\tau_1$ ,  $\tau_2$ ,  $F_1$ ,  $F_2$ , and  $G$ . The numerical values of the parameters are listed in Table 1 and seem reasonable.

### DISCUSSION

This analysis does not lead to values for the rate constants,  $k_i$ s, without additional data because the values of  $k_i$  only enter into the expression

$$A_i = c_i k_{1,i} + k_{2,i} \quad [8]$$

and  $k_{1,i}$  and  $k_{2,i}$  values occur in the slope and the intercept of the plot of  $A_i$  against  $c_i$  at the same pH. The pH dependence of  $k_{1,i}$  and  $k_{2,i}$  could be checked experimentally by varying the pH of the solution. A straight line for  $A_i$  plotted against  $c_i$  indicates that  $k_{1,i}$  and  $k_{2,i}$  are independent of the pH of the solution. We assume the value of  $c_i$  is not significantly changed by the depression of the nerve action potential because it is the bulk concentration of local anesthetics.

For any acid,  $(RH^+)$ ,

$$pK_a = pH + \log [RH^+]/[R] \quad [9]$$

and local anesthetic activity relates to the ratio of  $[RH^+]/[R]$ . Procaine has a  $pK_a$  of 8.95, which is greater than that of lidocaine, 7.85. When the pH is kept constant, the quantity  $[RH^+]/[R]$  increases with the increase in the value of  $pK_a$ .

It is believed that the potency of a local anesthetic is roughly proportional to its ability to penetrate neutral lipid (1). The pH of the solution determines the degree of this penetration, which is believed mostly to be due to the uncharged molecules.

Table 1. Parameters of Eq. 6

Local anesthetic	pH	$A_1$	$\tau_1$	$F_1$	$A_2$	$\tau_2$	$F_2$	$G$
Lidocaine	9.2	$4.765 \times 10^{-2}$	20.68	$6.933 \times 10^{-3}$	$9.463 \times 10^{-4}$	167.50	$9.927 \times 10^{-5}$	79.76
Lidocaine	7.2	$6.664 \times 10^{-1}$	5.07	$2.132 \times 10^{-2}$	$9.852 \times 10^{-2}$	34.06	$2.515 \times 10^{-3}$	56.28
Procaine	9.2	$2.752 \times 10^{-1}$	11.76	$1.049 \times 10^{-2}$	$2.317 \times 10^{-1}$	9.92	$9.981 \times 10^{-3}$	41.17
Procaine	7.2	$9.508 \times 10^{-1}$	4.47	$9.387 \times 10^{-3}$	$2.507 \times 10^{-1}$	12.95	$1.039 \times 10^{-2}$	87.37

There are at least two species that are involved in the depression of the action potential of the frog sciatic nerve. The two species are more active in procaine than in lidocaine. The time constants,  $\tau$ , are longer for lidocaine than for procaine.

The above findings lead to the following considerations. Assuming that the rate constants  $k_{1,i}$  and  $k_{2,i}$  of reaction 1 are not significantly affected by the pH drop,  $c_1$  and  $c_2$  increase with  $A_1$  and  $A_2$  according to Eq 8. The symbols,  $c_1$  and  $c_2$ , represent the concentrations of the inhibited and the uninhibited sites, respectively. When the total concentration of local anesthetic is not changed during the experiment, the concentration of the charged form increases inversely with that of the uncharged form during the pH drop. The addition of hydrogen ion converts R to  $RH^+$ , and although  $RH^+$  is more effective than R in the inhibition of nerve activity when present in the membrane,  $RH^+$  makes a weaker bond with the site than R. This is expressed by the consequent time-dependent decrease in the inhibition.

From the finding that  $\tau$  values decrease when pH drops from 9.2 to 7.2 and that procaine is more active than lidocaine, we may conclude that the charged form is the more active form of lidocaine and procaine. However, as stated above,  $RH^+$  is a weakly bonding inhibitor.

The procedure developed here should be generally useful.

### BLOCKING OR STIMULATION OF NERVOUS SYSTEM BY DRUGS

We next consider the effect on nerve function from the combining of the receptors, S, with drugs A and B. Let ( $S_0$ ) be the total number of such receptors and ( $S$ ) the number of receptors uncombined with drugs. We consider the case where both drugs A and B have been administered. Then

$$(S_0) = (S) + (SA_r) + (SB_t) + (SA_n B_m) \quad [10]$$

Making use of the various equilibria of which the following is typical:

$$S + rA = SA_r \quad [11]$$

$$\frac{(SA_r)}{(S)(A)^r} = K \quad [12]$$

We can rewrite [10] as

$$(S_0) = (S) + K_1(S)(A)^r + K_2(S)(B)^t + K_{12}(S)(A)^r(B)^m \quad [13]$$

If we let  $V$  be the size of the action potential without drugs and  $V_A$ ,  $V_B$ , and  $V_{AB}$  be the size of the action potential with the anesthetics, indicated by subscripts, then:

$$V = bk_0(S_0) \quad [14]$$

and

$$V_{AB} = b[k_0(S) + k_1K_1(S)(A)^r + k_2K_2(S)(B)^t + k_{12}K_{12}(S)(A)^r(B)^m] \quad [15]$$

Here  $k_0(S)$ ,  $k_1(SB_r)$ ,  $k_2(SB_t)$ , and  $k_{23}(SA_n B_m)$  are the several contributions to increasing the voltage  $V_{AB}$ . Dividing  $V$  by  $V_{AB}$ , using equation 13 to replace ( $S_0$ ), and then dividing numerator

and denominator by  $k_0(S_0)$  we have:

$$\frac{V}{V_{AB}} = \frac{1 + K_1(A)^r + K_2(B)^t + K_{12}(A)^r(B)^m}{1 + \frac{k_1}{k_0}K(A)^r + \frac{k_2}{k_0}K_2(B)^t + \frac{k_{12}}{k_0}K_{12}(A)^r(B)^m} \quad [16]$$

Subtracting 1 from both sides of the equation we have

$$\begin{aligned} \frac{V}{V_{AB}} - 1 &= \frac{K_1(A)^r \left(1 - \frac{k_1}{k_0}\right) + K_2(B)^t \left(1 - \frac{k_2}{k_0}\right) + K_{12}(A)^r(B)^m \left(1 - \frac{k_{12}}{k_0}\right)}{1 + \frac{k_1}{k_0}K(A)^r + \frac{k_2}{k_0}K_2(B)^t + \frac{k_{12}}{k_0}K_{12}(A)^r(B)^m} \end{aligned} \quad [17]$$

There are a number of interesting cases to be considered. *Case I:* If  $k_1 = k_2 = K_{12} = 0$ , we have

$$\frac{V}{V_{AB}} - 1 = \frac{V}{V_A} - 1 + \frac{V}{V_B} - 1 \quad [18]$$

In this case we have simple summation of anesthetic effects.

*Case II:* Let  $k_1 = k_2 = 0$  and  $k_{13} \gg k_0$  and  $k_{12}$  be large so that the numerator becomes large and negative. We then have  $(V)/(V_{BC}) \rightarrow 0$ , so that adding the two drugs causes great excitation. By adding the drugs singly we can have either excitation or depression, depending on the relative sizes of  $k_0$  with respect to  $k_1$  or  $k_2$ .

*Case III:* Suppose  $k_1 = k_2$  and  $k_{12}/k_0 K_{12}(A)^r(B)^m$  is a small term, then

$$\begin{aligned} \frac{V}{V_{AB}} - 1 &= \frac{V}{V_A} - 1 + \frac{V}{V_B} - 1 \\ &+ K_{12}(A)^r(B)^m \left(1 - 2\frac{k_{12}}{k_0}\right) \end{aligned} \quad [19]$$

and we have synergism or antagonism of the anesthetics depending on whether  $1 - (2k_{12}/k_0)$  is greater than or less than 1.

Other cases can be readily identified by considering Eq 17. This should help to clarify synergism, summation, and antagonism in anesthesia and nervous excitation.

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